RESPONSE AND AMENDMENT

In response to the Office Action of September 27, 2001, paper no. 37, please enter and consider the following amendments and remarks in connection with the above-identified application.

IN THE CLAIMS:

The following claims have been amended as indicated in a marked-up copy attached hereto:

Please cancel claims 4, 8, 9, 23, 35, and 41-43.

1. (Four times amended) An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising

[(a)] an essential gene, wherein expression of the essential gene in the cell is essential to the viability of the cell, and wherein said [the] essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment [, and wherein the essential gene is a copy of a wild-type gene of the microbial cell; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the wild-type gene is inactivated in the cell] , and wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell.

27. (Four times amended) A method of making a cell strain with environmentally limited viability comprising stably introducing into a cell

[(a)] an essential gene, wherein expression of the essential gene in the cell is essential to the viability of the cell, and wherein said [the] essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, [and wherein the essential gene is a copy of a wild-type gene of the microbial cell;

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment],

and wherein the cell strain is viable in a permissive environment and non-viable in a non-permissive environment [, wherein the wild-type gene is inactivated in the cell].

30. (Five times amended) A method of inducing immunoprotection in a warm-blooded animal comprising

administering to the animal [a vaccine comprising] a microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising

[(a)] an essential gene, wherein expression of the essential gene in the cell is essential to the viability of the cell, and wherein said [the] essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal [, and wherein the essential gene is a copy of a wild-type gene of the microbial cell; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal,

wherein the wild-type gene is inactivated in the cell],

and wherein the cell is a member of the *Enterobacteriaceae*.

46. (New) The cell of claim 1 further comprising a lethal gene, wherein expression of the lethal gene is lethal to the cell and wherein the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment.

47. (New) The cell of claim 46 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

48. (New) The cell of claim 46 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal,

wherein the cell is a member of the *Enterobacteriaceae*.

49. (New) The cell of claim 46 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector.

50. (New) The cell of claim 49 wherein the vector has two lethal genes.

51. (New) The cell of claim 46 wherein expression of the essential gene is regulated by the expression product of a regulatory gene.

52. (New) The cell of claim 51 wherein the expression product of the regulatory gene inhibits expression of the essential gene and said expression product is expressed or active only in the non-permissive environment.

53. (New) The cell of claim 49 wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed in the permissive environment and is not expressed in the non-permissive environment.

54. (New) The cell of claim 46 further comprising an expression gene wherein the expression gene encodes a desired expression product.

55. (New) The cell of claim 46 [for use as a vaccine], wherein the cell is viable when in an animal and non-viable when outside of the animal, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, and the lethal gene is expressed when the cell is outside of the animal and is not expressed when the cell is in the animal, wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C, wherein the cell is a member of *Enterobacteriaceae*.

56. (New) The cell of claim 55 further comprising an expression gene wherein the expression gene encodes a desired expression product.

57. (New) The method of claim 27 further comprising stably introducing into a cell a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment.

58. (New) The method of claim 57 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

59. (New) The method of claim 57 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal, wherein the cell is a member of *Enterobacteriaceae*.

60. (New) The method of claim 30, wherein said microbial cell further comprises [further comprising] a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal.

61. (New) The method of claim 60 wherein the system further [comprising] comprises an expression gene wherein the expression gene encodes an antigen.

62. (New) The method of claim 61 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

63. (New) The method of claim 60 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector, and wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for

replication of the vector, wherein the replication gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal,

wherein the cell is a member of the *Enterobacteriaceae*.

64. (New) The cell of claim 51 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the essential gene and wherein the expression product is not expressed or is inactive only in the permissive environment.

65. (New) The method of claim 46 wherein the essential gene is an *asd* gene, a *dap* gene, a *dal* gene, a *ddl* gene, a *fab*, gene, or a *pls* gene.

66. (New) An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, wherein said essential gene is native to the cell, and wherein said essential gene is inactivated in the cell;

(b) a copy of said essential gene, wherein said copy is introduced into the cell, and wherein said copy is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment; and

(c) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell.

67. (New) The cell of claim 66 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

68. (New) The cell of claim 66 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal, wherein the cell is a member of the *Enterobacteriaceae*.

69. (New) The cell of claim 66 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector.

70. (New) The cell of claim 69 wherein the vector has two lethal genes.

71. (New) The cell of claim 66 wherein expression of the essential gene is regulated by the expression product of a regulatory gene.

72. (New) The cell of claim 71 wherein the expression product of the regulatory gene inhibits expression of the essential gene and wherein said expression product is expressed or active only in the non-permissive environment.

73. (New) The cell of claim 69 wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed in the permissive environment and is not expressed in the non-permissive environment.

74. (New) The cell of claim 66 further comprising an expression gene wherein the expression gene encodes a desired expression product.

75. (New) The cell of claim 66 [for use as a vaccine], wherein the cell is viable when in an animal and non-viable when outside of the animal, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, and the lethal gene is expressed when the cell is outside of the animal and is not expressed when the cell is in the animal, wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C, wherein the cell is a member of *Enterobacteriaceae*.

76. (New) The cell of claim 75 further comprising an expression gene wherein the expression gene encodes a desired expression product.

77. (New) A method of making a cell strain with environmentally limited viability comprising

(a) inactivating an essential gene in a cell, wherein expression of the essential gene in the cell is essential to the viability of the cell, and wherein said essential gene is native to the cell; and

(b) stably introducing into the cell

(i) a copy of said essential gene, wherein said copy is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment; and

(ii) a lethal gene, wherein expression of the lethal gene is lethal to the cell and wherein the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell.

78. (New) The method of claim 77 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

79. (New) The method of claim 77 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal,

wherein the cell is a member of *Enterobacteriaceae.*

80. (New) A method of inducing immunoprotection in a warm-blooded animal comprising

administering to the animal [a vaccine comprising] a microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, wherein said essential gene is native to the cell, and wherein said essential gene is inactivated in the cell;

(b) a copy of said essential gene, wherein said copy is introduced into the cell, and wherein said copy is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal; and

(c) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal,

wherein the cell is a member of the *Enterobacteriaceae*.

81. (New) The method of claim 80 wherein the system further [comprising] comprises an expression gene wherein the expression gene encodes an antigen.

82. (New) The method of claim 81 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

83. (New) The method of claim 80 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector, and wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal,

wherein the cell is a member of the *Enterobacteriaceae*.

84. (New) The cell of claim 51 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the essential gene and wherein the expression product is not expressed or is inactive only in the permissive environment.